

§Appl. No. 10/009,500
Amdt. dated July 27, 2005
Reply to Office Action of, June 15, 2005

Listing of Claims:

Please **amend** the claims as follows:

Listing of Claims:

Claim 1 (Previously Presented) A purified protein isolated from the leech species *Hirudinaria manillensis* having the biological activity of a hyaluronidase which is not influenced in its activity by heparin, characterized in that it has a molecular weight of 53 – 60 kD dependent on glycosylation.

Claim 2 (Previously Presented) A glycosylated protein according to claim 1 having a molecular weight of 58 (± 2) kD.

Claim 3 (Previously Presented) A non-glycosylated protein according to claim 1 having a molecular weight of 54 (± 2) kD.

Claim 4 (Previously Presented) A protein according to claim 1 having an isoelectric point of 7.2 - 8.0.

Claim 5 (Previously Presented) A protein according to claim 1 having the amino acid sequence given in Fig. 7 (SEQ ID No. ~~1~~NO:1)

Claim 6 (Previously Presented) A protein according to claim 1 having a specific enzymatic activity of > 100 kU / mg protein.

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Claim 7 (Previously Presented) A process for isolating and purifying the protein of claim 1 comprising:

- (i) homogenization of heads of leeches of the species *Hirudinaria manillensis* with an acid buffer and centrifugation,
- (ii) ammonium sulfate precipitation of the supernatant of step (i),
- (iii) cation exchange chromatography,
- (iv) concanavalin A affinity chromatography,
- (v) hydrophobic interaction chromatography,
- (vi) affinity chromatography on matrices coated with hyaluronic acid fragments, and
- (vii) gel permeation chromatography.

Claim 8 (Previously Presented) A protein having the biological activity of a hyaluronidase which is not influenced in its activity by heparin and having a molecular weight of 53 – 60 kD dependent on glycosylation, obtainable by the process steps of claim 7.

Claim 9 (Original) A protein according to claim 8 having a specific enzymatic activity of > 100 kU / mg protein.

Claim 10 (Original) A DNA sequence coding for a protein of claim 1.

Claim 11 (Previously Presented) A DNA sequence coding for a protein of claim 8 comprising a nucleotide sequence depicted in Fig. 8 (SEQ. ID NO: 2) , Fig. 9 (SEQ. ID NO: 4) or Fig.10 (SEQ ID NO: 6).

Claim 12 (Original) A recombinant protein having the biological activity of a hyaluronidase encoded by any a DNA sequence of claim 11.

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Claim 13 (Currently Amended) A recombinant protein with the biological activity of a hyaluronidase and a molecular weight of 55 – 59 kD dependent on glycosylation having an amino acid sequence with at least 80% 94% homology to SEQ ID NO:3.

Claim 14 (Original) An expression vector comprising a DNA sequence of claim 10.

Claim 15 (Previously Presented) A host cell suitable for the expression of a protein of claim 12 which was transformed with a vector comprising a DNA sequence for a protein comprising a nucleotide sequence depicted in Fig. 8 (SEQ. ID NO: 2), Fig. 9 (SEQ. ID NO: 4) or Fig. 10 (SEQ ID NO: 6).

Claim 16 (Previously Presented) A pharmaceutical composition comprising a protein of claim 1 and a pharmaceutically acceptable diluent, carrier, or excipient therefor.

Claim 17 (Previously Presented) A pharmaceutical composition comprising the protein of claim 22 and a pharmaceutically acceptable diluent, carrier or excipient therefor.

Claim 18 (Cancelled)

Claim 19 (Previously Presented) A pharmaceutical composition according to claim 16, further comprising heparin.

Claim 20 (Previously Presented) A method of treating myocardial, cardiovascular and thrombotic disorders and tumors in a subject, comprising administering to a ~~subject in need of~~ a protein of claim 1 to said subject.

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Claim 21 (Previously Presented) A process of claim 7, further comprising:
(viii) enzymatic or chemical de-glycosylation of the purified protein.

Claim 22 (Previously Presented) A protein of claim 1 having the amino acid sequence of
SEQ ID NO: 3.

Claim 23 (Previously Presented) A protein of claim 1 having the amino acid sequence of
SEQ ID NO: 5.

Claim 24 (Previously Presented) A protein of claim 1 having the amino acid sequence of
SEQ ID NO: 7.

Claim 25 (Cancelled)

Claim 26 (Previously Presented) A protein of claim 13, having at least 96% homology to
SEQ ID NO: 3.

Claim 27 (Previously Presented) A protein of claim 13, having at least 97% homology to
SEQ ID NO: 3.